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Water Stress Alleviation Effects of Biostimulants on Greenhouse-Grown Tomato Fruit

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Abstract: The aim of the present study was to evaluate the effects of three biostimulant products (Nomoren (N), Twin Antistress (TW), x-Stress (XS) and control treatment (C: no biostimulants added)) on the nutritional value, chemical composition and bioactive properties of greenhouse tomato fruit grown under full (W+: 100% of field capacity) and deficit irrigation (W–: 70% of field capacity) conditions. Fat content was the highest for the fully irrigated plants that received no biostimulants (CW+), while proteins and carbohydrates and energetic value were the highest in the XSW+ treatment. The content of the main detected sugars (fructose, glucose and trehalose) varied depending on the irrigation and biostimulant treatment. The highest amounts of individual and total organic acids and tocopherols were recorded in fully irrigated plants treated with Twin Antistress (TW), whereas the lowest overall values were observed under deficit irrigation for plants that received the XS treatment. The most abundant fatty acids were palmitic (27.5–36.0%) and linoleic acid (27.4–35.4%), followed by oleic (9.2–21.2%), linolenic (5.4–13.1%) and stearic acid (5.3–6.8%). Moreover, the highest values of β-carotene and lycopene were recorded for the CW- and NW+ treatments, respectively. The TWW+ showed the highest antioxidant activity for both assays tested (TBARS and OxHLIA). Most of the tested extracts showed lower antibacterial activity against the tested bacteria compared to the positive controls. On the other hand, CW+, XSW+ and XSW- treatments showed higher antifungal activity (MIC values) than positive controls. In conclusion, each biostimulant product had a different effect on the determined characteristics depending on the level of irrigation. Therefore, more research is needed to better identify the mechanisms of action and the physiological processes, after which the tested biostimulants may be used to standardize the application of such products in tomato cultivation.

Keywords: *Solanum lycopersicum* L.; deficit irrigation; fruit quality; bioactive properties; free sugars; antioxidant activity; organic acids; antimicrobial properties

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1. Introduction

Population growth has rapidly increased the need for food, and the production of vegetable products is under pressure to adequately address global food security [1]. Despite a significant boost in crop yields from the intensification of horticultural cropping systems, farming practices have led to serious environmental impacts, such as deforestation, soil erosion, industrial pollution, declining surface and groundwater quality and biodiversity loss (including genetic erosion) [2]. To address these negative impacts, farming practices have to shift from conventional to more sustainable alternatives, aiming to find the best

compromise of high crop yield and final product quality and to achieve the reasonable use of natural resources such as irrigation water [3,4].

Nowadays, agriculture is one of the key sectors affected by drought, since the availability of irrigation water is becoming lower. The impact of drought on crop productivity and yield has severe consequences for food security and the economies of the affected world regions [5]. In particular, the scarcity of irrigation water in the semi-arid and arid regions of the Mediterranean basin is becoming a major barrier for crop production under the changing climate conditions. Special focus is given to the production of vegetables due to high yield components, but they have high irrigation requirements compared to other crops and are greatly affected by water shortage conditions [6]. To address the problem of drought and increase crops' resilience, various strategies are implemented, including specialized crop inputs, traditional crop breeding and genetic modification to alleviate drought stress effects. Scientific research focuses on a better understanding of the drought resistance of crops, aiming to reveal those protective mechanisms that operate at a morphological and physiological level [7–10].

A promising and environmentally friendly agronomic innovation is the use of natural plant biostimulants (PBs), which may enhance flowering, plant growth, fruit set, crop productivity and nutrient use efficiency (NUE), while they are also able to improve plant tolerance against a wide range of biotic and abiotic stressors [11,12]. Therefore, they could be used as a sustainable tool to mitigate the adverse effects of climate change through the increased resilience of crops to abiotic stressors [13]. Considering that biostimulant products are derived from a wide variety of sources, including the processing of food by-products, their implementation in modern agriculture has also an impact on the circular economy and may also increase the added value of crops [14–16]. Therefore, sustainable agriculture has turned its attention to the use of beneficial microorganisms as the key ingredients in biostimulant products [17,18]. Fungi have the ability to interact with the plant through the roots in various ways, ranging from mutualistic symbioses to parasitism [19,20]. In particular, mycorrhizal fungi are a heterogeneous group of taxa that establish symbioses with over 90% of all plant species and find numerous applications in modern horticulture [21–23]. Moreover, the application of biostimulants obtained from plant or seaweed extracts, as well as from the residues of crops, has shown positive results in plant growth as well as in plant adaptation to stress [24,25].

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops around the world and is particularly sensitive to a number of environmental stressors, including drought. It is often utilized as a model crop for plant growth studies, due to its ease of propagation, wide genetic variability and the short life cycle of plants [26]. Tomato fruit are also very popular in many processed forms, e.g., ketchup, canned as a whole or in pieces, puree, sauce, soup, juice or sundried, as well as fresh fruit. In addition to their culinary role in the human diet, tomatoes represent a food source with low energetic value and with unique constituents that may positively affect health, since they are rich in vitamins, minerals, lycopene, β-carotene and anticancer agents [7,27]. The use of biostimulants in tomato crops is becoming popular, since positive results have been observed in terms of yield and fruit quality, especially under abiotic stress conditions, where plants showed increased resilience [28–32]. For example, the foliar application of a plant-based biostimulant containing flavonoids and organic acids on tomato plants grown under stress conditions showed protective effects on the functions of the photosynthetic machinery [33]. Francesca et al. [34] also suggested the promoting effects of protein hydrolysates on hormonal biosynthesis in tomato plants grown subjected to heat, drought or combined stress. Other researchers reported the upregulation of genes involved in physiological functions (e.g., cell homeostasis, carbohydrate translocation and metabolism and stomatal closure, nutrient metabolism, osmotic regulation) through the application of biostimulants containing magnesium and polyphenols [35] or calcium [36].

Therefore, the objective of the present study was to investigate the effects of three biostimulant products compared to a control treatment (no biostimulant application) on

tomato chemical composition and fruit quality in relation to water deficit irrigation. The results of this study could be useful in implementing innovative cropping strategies in horticultural crops that are susceptible to drought stress, aiming to retain high yields and improved fruit quality while achieving sustainable irrigation water management.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiment was carried out at the experimental farm of the University of Thessaly in Velestino, Greece. Tomato plants (*Solanum lycopersicum* L. cv. Merilia) were purchased from a commercial nursery in seed trays, and seedlings were transplanted directly in soil on 22 March 2020 (35 days after sowing). After crop installation (60 days after transplantation; 22 May 2020), two levels of irrigation were applied, namely full (100% of field capacity) and deficit irrigation (70% of field capacity), while, in the intervening period between stress initiation and transplantation, all plants received full irrigation. Irrigation was applied via a drip irrigation system. Throughout the growing period, all plants were fertigated twice a week with a nutrient solution containing N-P-K at a rate of 330-330-330 ppm and according to the irrigation schedule. This formula was retained until fruit setting, and then the composition of the formula changed to 165-165-330 ppm (N-P-K) + 300 ppm Ca when the first fruit cluster was formed and until the end of the experimental period. To avoid differences in the nutritional status of plants, an extra tank with water was used to provide the additional amounts of water needed to achieve the desired field capacity level. Crop was arranged in rows 0.8 m apart, while the plant distance within each row was 0.5 m (approximately 25,000 plants/hectare).

Plants received three biostimulant treatments (plus a control treatment where no biostimulants were applied), which were randomly arranged within each row, following the split-plot factorial design, with irrigation level being the main plot and biostimulant treatments the subplots. Each treatment was replicated three times, while each replicate included 6 plants (144 plants in total). The biostimulant treatments included: (1) control (C: no biostimulants added), (2) Twin Antistress (TW) (contains natural microorganisms based on Bacillus subtilis and yeast and *Ascophyllum nodosum* extracts, as well as N (organic): 1%, organic carbon: 10%, and organic matter (<50 kDa): 30%); (3) x-Stress (XS) (contains 0.5% Cu, 2.0% Fe, 1.0% Mn and 2.0% Zn, all chelated with glycine); and (4) Nomoren (contains 20% of arbuscular mycorrhizal fungi (AMF) (*Glomus* spp.)). The detailed composition of each product has been previously described by our team [37–39]. Nomoren was provided by Anthis S.A., Greece; Twin Antistress was provided by Microspore Hellas—Sacom Hellas, Greece and x-Stress was provided by Agrofarm S.A., Greece. The application of biostimulants was carried out manually, three times throughout the growing period, through root irrigation, with 15-day intervals between each application. The first application started one day after water stress initiation (61 days after transplantation; 22 May 2022). Samples of fruit for chemical analyses were collected from the 5th truss of fruit, on 10 July 2022. Immediately after harvest, fruit were washed with distilled water, dried with soft paper and then were cut into pieces and placed under freezing conditions in air-sealed food bags. The frozen samples were lyophilized and then ground into powder and stored in deep freezing conditions until chemical analyses took place.

2.2. Chemical Analyses

2.2.1. Nutritional and Energetic Compound Determination

Samples of fresh fruit were stored at deep freezing conditions (−80 °C) and later freeze-dried before the chemical composition analysis. The determination of the nutritional and energetic values was carried out according to the procedures described by the Association of Official Analytical Chemists [40]. Crude fat content was estimated using a Soxhlet apparatus (Behr Labor Technik, Dussedolf, Germany) by extraction with petroleum ether. Protein content was determined according to the macro-Kjeldahl method (N x 6.25) using an automatic distillation and titration unit (model Pro-Nitro-A, JP Selecta, Barcelona, Spain)

and the ash composition of the samples was evaluated by incineration at 600 ± 15 °C. Total carbohydrate content was evaluated by the difference based on the following equation: total carbohydrates (g/100 g dry weight (dw)) = 100 − (g fat + g ash + g protein). Lastly, energetic values were estimated according to the Atwater system using the following equation: energy (kcal/100 g dw) = 4 × (g proteins + g carbohydrates) + 9 × (g fat).

2.2.2. Analysis of Free Sugars and Organic Acids

The free sugar composition was determined by high-performance liquid chromatography (HPLC) coupled to a refraction index (RI) detector, by means of the internal standard (IS, melezitose; Sigma-Aldrich, St. Louis, MO, USA) method, as previously defined [41]. The identification was performed by comparing the retention times of the authentic standards with those of the samples, whereas quantification was achieved by the IS method, with calibration curves built with the standards. The results were stated in g per 100 g of dw. The organic acid profile was identified by ultra-fast liquid chromatography (UFLC; Shimadzu 20A series, Kyoto, Japan) through a procedure previously labelled and optimized by the authors [42]. Detection was done in a photo-diode array detector (PDA), using 215 nm and 280 nm as preferable wavelengths. Quantification was completed by likening the peak area of the samples to calibration curves produced with commercial standards and was expressed in g per 100 g of dw.

2.2.3. Analysis of Lipophilic Compounds

The fatty acid methyl esters (FAME) profile was achieved after trans-esterification of the lipid fraction attained by Soxhlet extraction [41], followed by gas–liquid chromatography with flame ionization detection, using a YOUNG IN Crhomass 6500 GC System apparatus equipped with a split/splitless injector, a flame ionization detector (FID) and a Zebron-Fame column. Identification and quantification were completed by associating the relative retention times of the FAME peaks of the samples with those of the standard (47885-U; Sigma-Aldrich, St. Louis, MO, USA). The Clarity DataApex 4.0 Software (DataApex, Prague, Czech Republic) was utilized for data handing. The results were expressed as the relative percentage (%) of each detected fatty acid. Tocopherols were characterized following an analytical procedure previously described by the authors [41]. An HPLC system coupled to a fluorescence detector (FP-2020; Jasco) programed for excitation at 290 nm and emission at 330 nm was utilized. The isoform identification was attained by chromatographic comparison with authentic standards, and the quantification was founded on the fluorescence signal response of each standard, using the IS (tocol (50 mg/mL); Matreya, Pleasant Gap, PA, USA) method and calibration curves built with commercial standards. The results were expressed in mg per 100 g of dw.

2.2.4. Pigments

The content of carotenoids and chlorophylls was evaluated using a method described by the authors [43]. Briefly, the samples (500 mg) were vigorously shaken with 10 mL of acetone/hexane mixture (4:6, v/v) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance was measured at 453, 505, 645 and 663 nm, and the content of carotenoids (β-carotene and lycopene) and chlorophyll a and b was obtained with the following equations, and expressed in mg per 100 g of dw: β-carotene (mg/100 mL) = 0.216 × A663 − 1.220 × A645 − 0.304 × A505 + 0.452 × A453; lycopene (mg/100 mL) = −0.0458 × A663 + 0.204 × A645 − 0.304 × A505 + 0.452 × A453; chlorophyll a (mg/100 mL) = 0.999 × A663 − 0.0989 × A645; chlorophyll b (mg/100 mL) = −0.328 × A663 + 1.77 × A645.

2.3. Evaluation of Bioactive Properties In Vitro

2.3.1. Preparation of Hydroethanolic Extracts

The fresh fruit material was used to produce hydroethanolic extracts by stirring the plant material (~2.5 g) with 30 mL of ethanol/water (80:20, v/v) at 25 °C for 1 h and filtering it through Whatman No. 4 paper. The deposit was then re-extracted with an

extra 30 mL of the hydroalcoholic mixture. The joint extracts were concentrated at 40 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and further lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA).

2.3.2. Antioxidant Activity Evaluation

The antioxidant activity was evaluated though two cell-based assays: the thiobarbituric acid reactive substances (TBARS) assay and oxidative hemolysis (OxHLIA) based on the procedures described by Lockowandt et al. [44]. Regarding the TBARS assay, the lipid peroxidation inhibition was determined based on the color concentration of malondialdehyde–thiobarbituric acid (MDA-TBA) and measuring its absorbance at 532 nm. The inhibition ratio (%) was determined according to the following formula: $[(A - B)] \times 100\%$, where A and B were the absorbance values of the sample solutions and the control. The results were expressed as EC_{50} (mg/mL), which presents the sample's concentration that provides 50% antioxidant activity. The antihemolytic activity was evaluated though the OxHLIA assay and the results were expressed as IC_{50} values, which is the extract concentration (μg/mL) needed to prevent the oxidative hemolysis of 50% of the erythrocytes for Δt of 60 min. The positive control used for the determination of the antioxidant activity was Trolox (Sigma-Aldrich, St. Louis, MO, USA).

2.3.3. Antimicrobial Activity Evaluation

Staphylococcus aureus (American Type Culture Collection, Manassas, VA, USA, ATCC 11632), *Bacillus cereus* (food isolate), *Listeria monocytogenes* (National Collection of Type Cultures, London, UK, NCTC 7973), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (clinical isolate) and *Salmonella typhimurium* (ATCC 13311) were selected to test the antibacterial activity of the extracts prepared above. For antifungal activity, six micromycetes were used, namely *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Penicillium verrucosum* var. cyclopium (food isolate), *Penicillium funiculosum* (ATCC 36839) and *Trichoderma viride* (Institute of Applied Microbiology, University of Tokyo, Japan, IAM 5061). The microdilution method was performed as previously described [45]. The minimum inhibitory, bactericidal and fungicidal concentrations (MICs, MBCs and MFCs) were assessed using the serial dilution technique in 96-well microtiter plates (Spektar, Čačak, Serbia). The used positive controls were E211 and E224 (Sigma-Aldrich, St. Louis, MO, USA), whereas the negative control was 5% dimethyl sulfoxide (DMSO).

2.4. Statistical Analysis

All chemical analysis assays were carried out in triplicate and the results were expressed as mean ± standard deviation using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). All statistical tests were performed using with the aid of Statgraphics 5.1. plus (Statpoint Technologies, Inc., Warrenton, VA, USA) and the results were obtained through the analysis of variance (two-way ANOVA). When significant differences were detected ($\alpha = 0.05$), means were compared with Tukey's HSD test.

3. Results and Discussion

The statistical analysis of the data showed a significant interaction between the tested factors (e.g., irrigation regime and biostimulants); therefore, the means from all the treatment combinations were compared simultaneously according to Tukey's HSD test.

The results of fruit nutritional value are presented in Table 1. Fat content was the highest for the fully irrigated plants that received no biostimulants (CW+), while proteins and carbohydrates and energetic value were the highest in the XSW+ treatment and ash content in the TWW- treatment. However, the same treatment (TWW-) recorded the lowest values of fat and energetic value, while the lowest content of proteins, ash and carbohydrates was recorded in NW+, TWW+ and CW+ treatments, respectively. Moreover, plants subjected to deficit irrigation with no biostimulants added recorded higher values of

protein and carbohydrate content and energetic value than the corresponding full irrigation treatment. Similar findings were observed for the XSW- (higher values of fat and ash content than the XSW+ treatment) and NW- treatments (higher values of fat, protein and ash content than the NW+ treatment). This varied response could be attributed to compositional differences between the tested biostimulant products, where the arbuscular mycorrhizal fungi (NW) and chelated minerals (XS) alleviated the water stress effects and retained fruit quality. Similar results were reported by Fernandes et al. [39], who tested the same biostimulants on common bean and suggested water-stress-mitigating effects of the tested biostimulants on nutritional value parameters such as fat, proteins and ash content. These results could be associated with the improved nutrient status of plants, since previous studies have established the positive role of arbuscular mycorrhizal fungi (AMF), bacteria and seaweed extracts on nutrient uptake [31,35,46–48]. Moreover, the application of biostimulants containing chelated metals improved specific nutritional aspects of tomato fruit under full irrigation, which could be considered as a sustainable tool to improve fruit quality. This finding could be associated with the improved availability of minerals or the protective effects of minerals such as Se against oxidative stress [49]. The same protective effects have also been attributed to *Ascophyllum nodosum* extracts, which improved the redox status and nutritional parameters of crops [37,39,50,51].

The main detected free sugar was fructose (10.96–13.00 g/100 g dw), followed by glucose (6.84–9.97 g/100 g dw) and trehalose (0.17–0.35 g/100 g dw), in amounts that varied depending on the irrigation and biostimulant treatment (Table 1). Our results indicate that, with the exception of the NW+ treatment, all the tested biostimulants increased or were similar to control levels of total sugar content under deficit irrigation, whereas the lowest overall values for individual and total sugar content were recorded for the fully irrigated plants that received the XS treatment. Petropoulos et al. [51] also identified fructose and glucose as the main detected sugars, although the range of the fructose-to-glucose ratio was lower than that observed in our study (1.008–1.13 compared to 1.21–1.60). Abou Chehade [15] also reported fructose and glucose as the only detected sugars in organically grown tomato fruit, while they further suggested the involvement of sugars as biotic elicitors that allow plants to overcome stress conditions. Although trehalose was detected in low amounts, Paul et al. [52] suggested it as one of the osmolytes that was upregulated under the foliar application of a biostimulant and discriminated treated from untreated plants grown under water deficit conditions. In the literature review conducted by Delorge et al. [53], it was suggested that trehalose biosynthesis is associated with stress tolerance to abiotic stressors, especially in cases were sucrose levels are low or not detected, as in our study. Other soluble sugars, such as fructose and glucose, may serve as osmoprotectants under stress conditions through the regulation of stress-related genes and as energy sources for plant defense mechanisms [54–57]—hence, the increased content of individual and total free sugars observed in the current study.

The most abundant compound detected was citric acid in amounts that ranged between 29.14 and 46.32 g/100 g dw, followed by malic (2.37–4.77 g/100 g dw) and oxalic acid (0.68–1.21 g/100 g dw), while traces of ascorbic acid were also present (Table 1). The highest amounts of individual and total organic acids were recorded in fully irrigated plants treated with TW, whereas the lowest overall values were observed under deficit irrigation for plants that received the XS treatment. This finding coincides with the report of Pishchik et al. [58], who suggested that the application of *Bacillus subtilis* had a beneficial effect on the organic acid content of tomato fruit. In general, organic acid content decreased under water stress for all the biostimulant treatments compared to the respective treatments of fully irrigated plants, which suggests the small contribution of organic acids as osmoprotectants under stress. This could also be manifested by the values of the total sugar/organic acid ratio, which increased under deficit irrigation, suggesting higher amounts of total sugars and better fruit quality [51]. In contrast to our study, Subramanian et al. [28] suggested an increase in titratable acidity under severe water stress, while they also reported a negative effect of mycorrhizal colonization on fruit acidity. On the other hand, Kalozoumis et al. [32]

did not observe a significant effect of biostimulant application and water and nutrient restriction on tomato fruit acidity, while Koleška et al. [30] reported a negative effect of biostimulant application on total soluble solids and total acidity in tomato fruit. Therefore, it could be assumed that such contradictory results could be attributed either to differences in stress severity and biostimulant product or to genotypic differences [32].

Table 1. Nutritional, energetic value and hydrophilic compounds of tomato fruit in relation to irrigation regime (W+/W-) and biostimulant treatment (C, TW, XS, N) (mean ± SD, n = 3).

	CW+	TWW+	XSW+	NW+	CW-	TWW-	XSW-	NW-
Nutritional Value				**(g/100 g dw)**				
Fat	4.53 ± 0.02 a	2.26 ± 0.04 e	2.08 ± 0.06 f	3.75 ± 0.07 d	4.41 ± 0.01 b	1.53 ± 0.01 g	3.94 ± 0.03 c	3.88 ± 0.01 c
Proteins	10.98 ± 0.09 e	11.90 ± 0.06 b	13.28 ± 0.08 a	10.42 ± 0.08 f	11.04 ± 0.04 e	11.22 ± 0.08 d	11.62 ± 0.02 c	11.50 ± 0.01 c
Ash	15.92 ± 0.09 b	12.41 ± 0.06 f	10.72 ± 0.07 g	14.36 ± 0.08 e	15.11 ± 0.08 c	17.82 ± 0.02 a	14.86 ± 0.09 d	14.84 ± 0.06 d
Carbohydrates	68.58 ± 0.01 f	73.43 ± 0.06 b	73.92 ± 0.05 a	71.46 ± 0.06 c	69.44 ± 0.06 e	69.44 ± 0.03 e	69.57 ± 0.07 e	69.78 ± 0.03 d
Energy (Kcal/100 g dw)	359.0 ± 0.2 d	361.6 ± 0.3 b	367.5 ± 0.4 a	361.3 ± 0.5 b	361.6 ± 0.2 b	336.4 ± 0.1 e	360.2 ± 0.1 c	360.1 ± 0.2 c
Free sugars				**(g/100 g dw)**				
Fructose	12.81 ± 0.04 b	12.56 ± 0.07 c	10.96 ± 0.01 g	13.00 ± 0.03 a	12.51 ± 0.07 c	12.22 ± 0.04 d	11.44 ± 0.02 f	12.05 ± 0.01 e
Glucose	9.02 ± 0.04 d	8.61 ± 0.05 e	6.84 ± 0.04 f	9.10 ± 0.05 d	9.75 ± 0.03 b	9.97 ± 0.07 a	9.43 ± 0.04 c	9.70 ± 0.04 b
Trehalose	0.32 ± 0.04 ab	0.19 ± 0.01 c	0.17 ± 0.04 c	0.21 ± 0.01 c	0.282 ± 0.007 b	0.31 ± 0.01 ab	0.34 ± 0.01 a	0.35 ± 0.02 a
Total	22.2 ± 0.1 bc	21.37 ± 0.03 d	17.97 ± 0.09 f	22.30 ± 0.07 b	22.54 ± 0.09 a	22.50 ± 0.04 a	21.21 ± 0.04 e	22.10 ± 0.05 c
Organic acids				**(g/100 g dw)**				
Oxalic acid	0.95 ± 0.01 c	1.21 ± 0.01 a	1.15 ± 0.01 b	0.77 ± 0.01 e	0.93 ± 0.01 d	0.72 ± 0.01 f	0.68 ± 0.01 g	0.71 ± 0.01 f
Malic acid	2.93 ± 0.01 c	4.77 ± 0.01 a	4.43 ± 0.06 b	2.94 ± 0.02 c	2.69 ± 0.03 d	2.47 ± 0.01 f	2.37 ± 0.01 g	2.59 ± 0.02 e
Ascorbic acid	tr *	tr	tr	tr	tr	tr	tr	tr
Citric acid	37.7 ± 0.6 c	40.34 ± 0.03 a	39.3 ± 0.4 b	30.45 ± 0.02 e	36.0 ± 0.8 d	28.4 ± 0.1 f	26.08 ± 0.02 g	28.29 ± 0.09 f
Total	41.6 ± 0.6 c	46.32 ± 0.03 a	44.9 ± 0.3 b	34.16 ± 0.01 e	39.7 ± 0.8 d	31.6 ± 0.1 f	29.14 ± 0.01 g	31.6 ± 0.1 f

* tr—traces; means in the same row followed by different Latin letters are significantly different according to Tukey's HSD test at p = 0.05.

The fatty acid composition in relation to the irrigation regime and biostimulant treatment is presented in Table 2. A total of seventeen individual compounds were detected in the studied samples. The most abundant fatty acids were palmitic (27.5–36.0%) and linoleic acid (27.4–35.4%), followed by oleic (9.2–21.2%), linolenic (5.4–13.1%) and stearic acid (5.3–6.8%), while the most abundant classes of fatty acids were saturated and polyunsaturated fatty acids (SFA: 38.5–47.5% and PUFA: 38.0–43.2%). Similar results were reported by Petropoulos et al. [51], who also suggested palmitic and linoleic as the main fatty acids, while they recorded a variable fatty acid profile depending on the fruit harvesting date. In the case of our study, a varied response was recorded for individual fatty acids' content in relation to biostimulant and irrigation treatment, with no specific trends being observed, while SFA and PUFA content increased under deficit irrigation and when plants were treated with biostimulant products. In contrast, MUFA content increased under full irrigation, regardless of the biostimulant treatment, mostly due to the increased content of oleic acid. According to the literature, unsaturated fatty acids represent one of the main defense systems of plants against abiotic and biotic stressors [59], which is in accordance with our findings, where the PUFA content increased under deficit irrigation for all the biostimulant treatments compared to fully irrigated plants that received full irrigation. Moreover, C18 unsaturated fatty acids such as linoleic and linolenic acid are usually utilized for the production of aliphatic compounds for cell membrane protection, an argument

that is also consistent with the findings of our study, where both compounds showed a decrease under deficit irrigation, regardless of the biostimulant treatment [59]. The values of the PUFA/SFA ratio were higher than 0.45 for all the tested treatments, indicating the high nutritional value of fruit [51]. On the other hand, the values of the n6/n3 ratio were below 4.0 when plants were grown under water deficit conditions, as well as in fully irrigated plants treated with the XS treatment, due to the increase in linoleic and under these conditions. This parameter is also important for the nutritional value of tomato fruit, and deficit irrigation could be a useful tool to improve the quality of fruit in a sustainable manner [60].

Table 2. Chemical composition with regard to lipophilic compounds (fatty acids and tocopherols) of tomato fruit in relation to irrigation regime and biostimulant treatment (mean ± SD, n = 3).

Fatty Acids	CW+	TWW+	XSW+	NW+	CW-	TWW-	XSW-	NW-
				Relative Percentage (%)				
C6:0	0.204 ± 0.003 ^{e}	0.44 ± 0.02 ^{a}	0.33 ± 0.01 ^{b}	0.28 ± 0.01 ^{c}	0.269 ± 0.001 ^{c}	0.306 ± 0.003 ^{b}	0.215 ± 0.003 ^{e}	0.246 ± 0.006 ^{d}
C8:0	0.063 ± 0.002 ^{c}	0.095 ± 0.004 ^{a}	0.096 ± 0.001 ^{a}	0.066 ± 0.004 ^{c}	0.084 ± 0.001 ^{b}	0.094 ± 0.001 ^{a}	0.054 ± 0.001 ^{d}	0.052 ± 0.001 ^{d}
C10:0	0.031 ± 0.001 ^{e}	0.078 ± 0.001 ^{b}	0.094 ± 0.005 ^{a}	0.064 ± 0.004 ^{c}	0.057 ± 0.003 ^{d}	0.070 ± 0.001 ^{b}	0.065 ± 0.004 ^{c}	0.059 ± 0.001 ^{d}
C12:0	0.065 ± 0.001 ^{e}	0.096 ± 0.005 ^{c}	0.102 ± 0.004 ^{b}	0.093 ± 0.008 ^{c}	0.092 ± 0.001 ^{c}	0.070 ± 0.001 ^{d}	0.131 ± 0.008 ^{a}	0.066 ± 0.001 ^{e}
C14:0	0.466 ± 0.003 ^{e}	0.358 ± 0.0006 ^{f}	0.71 ± 0.02 ^{c}	1.07 ± 0.06 ^{a}	0.61 ± 0.01 ^{d}	0.689 ± 0.004 ^{c}	0.829 ± 0.001 ^{b}	0.654 ± 0.007 ^{cd}
C15:0	0.249 ± 0.002 ^{f}	0.299 ± 0.007 ^{e}	0.37 ± 0.02 ^{b}	0.38 ± 0.03 ^{b}	0.390 ± 0.006 ^{a}	0.330 ± 0.001 ^{c}	0.376 ± 0.005 ^{b}	0.310 ± 0.007 ^{d}
C16:0	28.4 ± 0.5 ^{f}	27.53 ± 0.01 ^{g}	37.2 ± 0.2 ^{a}	33.1 ± 0.6 ^{e}	36.0 ± 0.1 ^{b}	35.5 ± 0.3 ^{c}	36.0 ± 0.3 ^{b}	34.5 ± 0.6 ^{d}
C16:1	0.759 ± 0.002 ^{e}	0.50 ± 0.02 ^{f}	1.14 ± 0.01 ^{c}	0.80 ± 0.07 ^{d}	1.77 ± 0.05 ^{a}	1.42 ± 0.02 ^{b}	1.746 ± 0.006 ^{a}	1.154 ± 0.003 ^{c}
C17:0	0.416 ± 0.004 ^{c}	0.34 ± 0.02 ^{e}	0.363 ± 0.001 ^{d}	0.37 ± 0.02 ^{d}	0.724 ± 0.005 ^{a}	0.592 ± 0.008 ^{b}	0.62 ± 0.02 ^{b}	0.563 ± 0.001 ^{b}
C18:0	6.06 ± 0.05 ^{c}	6.8 ± 0.3 ^{a}	5.3 ± 0.2 ^{e}	6.1 ± 0.1 ^{c}	6.03 ± 0.03 ^{c}	5.31 ± 0.01 ^{e}	6.49 ± 0.01 ^{b}	5.62 ± 0.02 ^{d}
C18:1n9c	17.2 ± 0.6 ^{b}	21.16 ± 0.05 ^{a}	12.76 ± 0.08 ^{d}	16.92 ± 0.31 ^{c}	9.2 ± 0.7 ^{g}	10.3 ± 0.1 ^{e}	10.3 ± 0.1 ^{e}	9.7 ± 0.2f
C18:2n6c	35.40 ± 0.02 ^{a}	34.4 ± 0.5 ^{b}	29.8 ± 0.1 ^{d}	30.7 ± 0.2 ^{c}	29.13 ± 0.02 ^{d}	28.5 ± 0.7 ^{e}	27.38 ± 0.03 ^{f}	29.9 ± 0.7 ^{d}
C18:3n3	7.81 ± 0.08 ^{d}	5.4 ± 0.1 ^{f}	8.95 ± 0.01 ^{c}	7.30 ± 0.09 ^{e}	13.1 ± 0.7 ^{a}	12.8 ± 0.2 ^{b}	13.1 ± 0.4 ^{a}	13.11 ± 0.05 ^{a}
C20:0	1.04 ± 0.01 ^{d}	0.776 ± 0.002 ^{f}	0.89 ± 0.01 ^{e}	0.70 ± 0.06 ^{g}	1.16 ± 0.01 ^{c}	1.27 ± 0.02 ^{b}	1.02 ± 0.01 ^{d}	1.55 ± 0.02 ^{a}
C22:0	0.69 ± 0.01 ^{d}	0.56 ± 0.03 ^{f}	0.69 ± 0.01 ^{d}	0.69 ± 0.01 ^{d}	0.587 ± 0.004 ^{e}	0.96 ± 0.04 ^{b}	0.831 ± 0.001 ^{c}	1.09 ± 0.01 ^{a}
C23:0	0.41 ± 0.01 ^{c}	0.339 ± 0.001 ^{d}	0.49 ± 0.01 ^{a}	0.41 ± 0.01 ^{c}	0.299 ± 0.006 ^{e}	0.44 ± 0.01 ^{b}	0.280 ± 0.001 ^{f}	0.455 ± 0.004 ^{b}
C24:0	0.71 ± 0.05 ^{f}	0.77 ± 0.06 ^{e}	0.812 ± 0.004 ^{d}	0.97 ± 0.08 ^{c}	0.524 ± 0.004	1.29 ± 0.01 ^{a}	0.563 ± 0.004 ^{g}	1.05 ± 0.05 ^{b}
SFA	38.8 ± 0.5 ^{d}	38.5 ± 0.4 ^{d}	47.4 ± 0.1 ^{a}	44.2 ± 0.6 ^{c}	46.86 ± 0.03 ^{ab}	47.0 ± 0.4 ^{ab}	47.5 ± 0.3 ^{a}	46.2 ± 0.7 ^{b}
MUFA	17.9 ± 0.6 ^{b}	21.65 ± 0.03 ^{a}	13.90 ± 0.08 ^{c}	17.72 ± 0.24 ^{b}	10.9 ± 0.7 ^{e}	11.8 ± 0.1 ^{d}	12.1 ± 0.1 ^{d}	10.81 ± 0.03 ^{e}
PUFA	43.2 ± 0.1 ^{a}	39.8 ± 0.4 ^{d}	38.7 ± 0.1 ^{e}	38.0 ± 0.3 ^{e}	42.2 ± 0.7 ^{b}	41.3 ± 0.5 ^{c}	40.4 ± 0.4 ^{cd}	43.0 ± 0.7 ^{ab}
Tocopherols				**(mg/100 g dw)**				
α-Tocopherol	11.67 ± 0.09 ^{c}	13.94 ± 0.01 ^{a}	12.02 ± 0.08 ^{b}	10.53 ± 0.06 ^{e}	10.97 ± 0.01 ^{d}	10.03 ± 0.07 ^{f}	9.24 ± 0.01 ^{h}	9.73 ± 0.09 ^{g}
γ-Tocopherol	4.49 ± 0.04 ^{c}	5.70 ± 0.01 ^{a}	4.99 ± 0.01 ^{b}	3.67 ± 0.03 ^{e}	4.10 ± 0.01 ^{d}	3.49 ± 0.01 ^{f}	3.14 ± 0.07 ^{h}	3.28 ± 0.01 ^{g}
Total	16.2 ± 0.1 ^{c}	19.65 ± 0.01 ^{a}	17.01 ± 0.07 ^{b}	14.20 ± 0.08 ^{e}	15.07 ± 0.01 ^{d}	13.53 ± 0.08 ^{f}	12.39 ± 0.07 ^{h}	13.01 ± 0.09 ^{g}

C6:0—caproic acid; C8:0—caprylic acid; C10:0—capric acid; C12:0—lauric acid; C14:0—myristic acid; C15:0—pentadecylic acid; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—margaric acid; C18:0—stearic acid; C18:1n9c—oleic acid; C18:2n6c – linoleic acid; C18:3n3 – linolenic acid; C20:0—arachidic acid; C22:0—behenic acid; C23:0—tricosylic acid; C24:0—lignoceric acid; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids. Means in the same row followed by different Latin letters are significantly different according to Tukey's HSD test at p = 0.05.

Regarding tocopherol composition, α- and γ-tocopherols were the only compounds detected in tomato fruit samples in our study (Table 2). Similar results were reported in the literature, with α- and γ-tocopherols being identified as the most abundant isoforms of vitamin E, whereas other tocopherols (β- and δ-tocopherol) were detected in lower amounts [51,61]. The highest and lowest content of individual and total tocopherols was recorded for the TWW+ and XSW- treatments, respectively, while water deficit irrigation resulted in lower content of tocopherols for all the tested biostimulants compared to the corresponding treatments under full irrigation. A similar response was recorded for the organic acid content, as previously described in Table 1. These findings coincide with the report of Fernandes et al. [39], who also recorded increased content of α-tocopherol in water-stressed common bean plants treated with TW, while the application of seaweed extracts has shown positive effects on the tocopherol content of tomato fruit in many reports [62]. In contrast, Chehade et al. [15] did not observe a significant effect of three potential biostimulants, although no stress conditions were implemented in their study. Considering that tocopherols are potent antioxidants [13], it could be assumed either that the tested water deficit conditions disrupted the specific protective mechanism that involves the biosynthesis of tocopherols, or that other antioxidants contributed to plant protection under the conditions of our study. Another possible explanation is the allocation of tocopherols mainly in the chloroplasts of leaves, where they serve as protectants of the photosynthetic system against stressors [63]; thus, their content in fruit is less affected under stress conditions.

The content of fruit pigments is presented in Table 3. Carotenoids consisted of β-carotene and lycopene, which showed varied content that ranged between 1.02 and 1.45 mg/100 g dw and between 4.27 and 4.61 mg/100 g dw for β-carotene and lycopene, respectively. In general, higher amounts of lycopene were recorded compared to β-carotene, a finding that coincides with previous reports, suggesting that lycopene is the main carotenoid of tomato fruit [51]. Moreover, the highest values of β-carotene and lycopene were recorded for the CW- and NW+ treatments, respectively, while the content of both compounds and total carotenoids either remained unchanged or decreased for the tested biostimulants under deficit irrigation compared to the fully irrigated plants under the same biostimulant treatments. The only exception was observed for the content of lycopene in the XSW- treatment, where an increase was recorded over the same treatment for full irrigation. These findings indicate a response that depended on the biostimulant as well as on the water status of plants, since carotenoids are considered a part of the non-enzymatic protective mechanism of plants [7]. According to literature reports, biostimulants consisting of protein hydrolysates, carbohydrates (paramylon) or seaweed extracts may increase the carotenoid content under optimal or unfavorable conditions [64,65], whereas other reports did not record any effects on the carotenoid content of organically grown tomato fruit after the application of various biostimulants obtained from food by-products [15] or based on humic substances [16]. Therefore, apart from the water status of plants (e.g., irrigation regime), there seems to be a biostimulant-specific response of carotenoid content. Regarding the chlorophyll content, no significant changes were observed in chlorophyll a, chlorophyll b and total chlorophylls in relation to the irrigation regime and biostimulant treatment applied. The only exception was recorded in the untreated plants that received full irrigation, where chlorophyll b and total chlorophyll content was significantly lower than under specific treatments (e.g., TWW+, CW- and XSW-). Several reports suggest the beneficial effects of microbial biostimulants on the leaf chlorophyll content of various vegetables [58,64,66–69], whereas, in fruit, this effect is expected to be lessened due to the gradual degradation of chlorophyll with ripening progression [70].

The results of the antioxidant activity of the tested tomato fruit samples are illustrated in Table 4. Contrasting results were observed between the two assays implemented for the determination of the antioxidant activity, while, in all cases, the observed activities were lower than in the positive control. In particular, the highest antioxidant activity for the TBARS assay was recorded for the TWW+ and XSW+ treatments, while the samples

with the highest activity for the OxHLIA assay were those obtained from the TWW+ and NW- treatments. On the other hand, the lowest activities were observed for treatments XSW- and NW- (TBARS assay) and CW+, NW+ and XSW- (OxHLIA assay). This variability could be explained by the different targeted compounds in each assay, as well as by possible synergistic or antagonistic effects between antioxidant compounds in tomato fruit [71,72]. According to the literature, microbial biostimulants (AMF and plant growth promoting rhizobacteria) did not show protective effects against lipid peroxidation damage in tomato shoots [73], whereas Campobenedetto et al. [74] and Goñi et al. [75] suggested that seaweed extracts increased the antioxidant activity in tomato leaves. In contrast, Abdel Latef et al. [76] reported that inoculation with *Glomus mossae* could increase the antioxidant activity in pepper plants and induce the protective mechanism against salt stress, while similar effects were observed for the foliar application of protein hydrolysates on tomato plants grown under optimal conditions [77]. Caruso et al. [78] suggested that the biostimulant application did not have a consistent effect on lipophilic and hydrophilic activity in tomato fruit, which is in agreement with the results obtained from the present study, while similar trends were reported by Parađiković et al. [79] and Murtic et al. [80] for pepper and tomato fruit, respectively.

Table 3. Carotenoid and chlorophyll composition (mg/100 g dw) of tomato fruit in relation to irrigation regime and biostimulant treatment (mean ± SD, n = 3).

	β-Carotene	Lycopene	Total Carotenoids	Chlorophyll [a]	Chlorophyll [b]	Total Chlorophylls
CW+	1.27 ± 0.07 ^{bc}	4.47 ± 0.02 ^{b}	5.73 ± 0.05 ^{a}	0.21 ± 0.01 ^{a}	0.15 ± 0.02 ^{b}	0.36 ± 0.01 ^{b}
TWW+	1.20 ± 0.08 ^{cd}	4.36 ± 0.02 ^{cd}	5.56 ± 0.05 ^{bc}	0.212 ± 0.008 ^{a}	0.19 ± 0.01 ^{a}	0.399 ± 0.009 ^{a}
XSW+	1.39 ± 0.07 ^{ab}	4.30 ± 0.03 ^{de}	5.69 ± 0.05 ^{ab}	0.21 ± 0.01 ^{a}	0.172 ± 0.004 ^{ab}	0.386 ± 0.025 ^{ab}
NW+	1.10 ± 0.09 ^{de}	4.61 ± 0.06 ^{a}	5.70 ± 0.07 ^{ab}	0.204 ± 0.001 ^{a}	0.16 ± 0.02 ^{ab}	0.365 ± 0.015 ^{ab}
CW-	1.45 ± 0.05 ^{a}	4.27 ± 0.03 ^{e}	5.73 ± 0.04 ^{a}	0.21 ± 0.01 ^{a}	0.19 ± 0.03 ^{a}	0.40 ± 0.02 ^{a}
TWW-	1.23 ± 0.05 ^{c}	4.33 ± 0.06 ^{de}	5.56 ± 0.05 ^{bc}	0.20 ± 0.02 ^{a}	0.17 ± 0.02 ^{ab}	0.37 ± 0.02 ^{ab}
XSW-	1.02 ± 0.05 ^{e}	4.44 ± 0.04 ^{bc}	5.45 ± 0.04 ^{c}	0.203 ± 0.001 ^{a}	0.19 ± 0.02 ^{a}	0.394 ± 0.015 ^{a}
NW-	1.10 ± 0.05 ^{de}	4.41 ± 0.06 ^{bc}	5.51 ± 0.05 ^{c}	0.21 ± 0.01 ^{a}	0.16 ± 0.01 ^{ab}	0.38 ± 0.01 ^{ab}

Means in the same column followed by different Latin letters are significantly different according to Tukey's HSD test at p = 0.05.

Table 4. Antioxidant activity of the studied tomato fruit hydroethanolic extracts in relation to irrigation regime and biostimulant treatment (mean ± SD, n = 3).

	TBARS (EC_{50}; mg/mL)	OxHLIA (IC_{50}; μg/mL) Δt = 60 min
CW+	0.73 ± 0.01 ^{e}	82 ± 3 ^{ab}
TWW+	0.60 ± 0.02 ^{g}	49 ± 1 ^{d}
XSW+	0.65 ± 0.03 ^{fg}	79 ± 3 ^{abc}
NW+	1.06 ± 0.09 ^{bc}	82 ± 2 ^{ab}
CW-	0.90 ± 0.01 ^{d}	75 ± 2 ^{bc}
TWW-	1.04 ± 0.04 ^{c}	73 ± 2 ^{c}
XSW-	1.21 ± 0.07 ^{a}	84 ± 5 ^{a}
NW-	1.1 ± 0.1 ^{ab}	48 ± 2 ^{d}
Positive control	0.0054 ± 0.003	21.8 ± 0.2

Means in the same column followed by different Latin letters are significantly different according to Tukey's HSD test at p = 0.05.

The antimicrobial (antibacterial and antifungal) properties of the tested tomato fruit extracts are presented in Tables 5 and 6. In particular, most of the tested extracts showed lower antibacterial activity (higher MIC and MBC values) against the tested bacteria compared to the positive controls (E211 and/or E224), especially against *Listeria monocytogenes*, *Escherichia coli* and *Enterobacter cloacae*, where E224 showed the highest activity, or against

Bacillus cereus, where E211 was the most efficient (Table 5). Finally, the extracts obtained from fruit under the CW+, TWW- and XSW- treatments had similar MIC values to the most potent positive control (E211) against *Staphylococcus aureus*, while TWW+ and CW- yielded the same MIC values as E224 against *Salmonella typhimurium*. On the other hand, specific extracts showed higher antifungal activity (MIC values) than positive controls in several cases, such as the extracts obtained from the CW+, XSW+ and XSW- treatments against *Aspergillus fumigatus*. Similar findings were recorded for all the extracts against *A. ochraceus*, regardless of biostimulant and irrigation treatment, or for all the extracts of fruit grown under full irrigation against *Penicillium verrucosum* var. *cyclopium*. High antifungal activity against *Trichoderma viride* was also observed for the extracts obtained from plants that received the treatments NW+ and NW- (Table 6). Finally, all the extracts showed similar MIC and lower MBC values compared to E224 against *A. niger* and *P. funiculosum*. Similar results were reported by Fernandes et al. [39], who evaluated the same biostimulants on common bean plants grown under deficit irrigation and suggested lower antibacterial and higher or similar antifungal activity compared to the positive controls for the pod extracts. The present findings do not show consistent results or trends for the tested extracts, indicating that the observed effects and differences among the extracts could be associated either with bioactive compounds in which water stress may induce biosynthesis, or to compounds that biostimulant products may contain *per se*. Such compounds could include polyphenols, as it is well known that their biosynthesis is induced under stress and they may exert antimicrobial activity [81–83], although this activity is associated with the phenolic skeleton and the position of the phenyl group [84]. On the other hand, other researchers suggest fatty acids as the compounds responsible for the antibacterial effects [85], although Ronga et al. [86] associated the antibacterial effects with the differences in cell wall structures between Gram-positive and Gram-negative bacteria. Moreover, oligosaccharides obtained from seaweed extracts could induce the biosynthesis of phenylpropanoids with antibacterial effects [87], while the aqueous extracts obtained from Baltic seaweeds after boiling also exerted antimicrobial effects due to the high content of phenolic compounds [88]. However, in the review report by Righini et al. [89], who used strawberry plants as a case study, the use of algae extracts as biostimulants showed a variable effect against plant pathogens.

Table 5. Antibacterial activity (minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC), mg/mL) of tomato fruit hydroethanolic extracts in relation to irrigation regime and biostimulant treatment.

		S. aureus (ATCC 11632)	*B. cereus* (Food Isolate)	*L. monocytogenes* (NCTC 7973)	*E. coli* (ATCC 25922)	*E. cloacae* (Clinical Isotated)	*S. typhimurium* (ATCC 13311)
CW+	MIC	1	1	2	1	1	2
	MBC	2	2	4	2	2	4
TWW+	MIC	2	1	2	1	1	1
	MBC	4	2	4	2	2	2
XSW+	MIC	2	1	2	1	2	2
	MBC	4	2	4	2	4	4
NW+	MIC	2	1	2	1	2	2
	MBC	4	2	4	2	4	4
CW-	MIC	2	1	2	1	1	1
	MBC	4	2	4	2	2	2
TWW-	MIC	1	1	2	1	1	2
	MBC	2	2	4	2	2	4
XSW-	MIC	1	1	2	1	2	2
	MBC	2	2	4	2	4	4
NW-	MIC	2	1	2	1	1	2
	MBC	4	2	4	2	2	4
E211 *	MIC	4	0.5	1	1	2	1
	MBC	4	0.5	2	2	4	2
E224	MIC	1	2	0.5	0.5	0.5	1
	MBC	1	4	1	1	0.5	1

* E211—sodium benzoate; E224—potassium metabisulfite.

Table 6. Antifungal activity (MIC and minimal fungicidal concentration (MFC), mg/mL) of tomato fruit hydroethanolic extracts in relation to irrigation regime and biostimulant treatment.

		A. fumigatus (Human Isolate)	*A. niger* (ATCC 6275)	*A. ochraceus* (ATCC 12066)	*P. v. var. Cyclopium* (Food Isolate)	*P. funiculosum* (ATCC 36839)	*T. viride* (IAM 5061)
CW+	MIC	0.5	1	0.5	0.25	0.5	1
	MFC	1	2	1	0.5	1	2
TWW+	MIC	1	1	0.5	0.25	0.5	1
	MFC	2	2	1	0.5	1	2
XSW+	MIC	0.5	1	0.5	0.25	0.5	1
	MFC	1	2	1	0.5	1	2
NW+	MIC	1	1	0.5	0.25	0.5	0.5
	MFC	0.5	2	1	0.5	1	1
CW-	MIC	1	1	0.5	0.5	0.5	1
	MFC	2	2	1	1	1	2
TWW-	MIC	1	1	0.5	0.5	0.5	1
	MFC	2	2	1	1	1	2
XSW-	MIC	0.5	1	0.5	0.5	0.5	1
	MFC	1	2	1	1	1	2
NW-	MIC	1	1	0.5	0.5	0.5	0.5
	MFC	2	2	1	1	1	1
E211 *	MIC	1	1	1	2	1	1
	MFC	2	2	2	4	2	2
E224	MIC	1	1	1	1	0.5	0.5
	MFC	1	1	1	1	0.5	0.5

* E211—sodium benzoate; E224—potassium metabisulfite.

4. Conclusions

Tomato is one of the most important vegetables worldwide, with high needs in irrigation water, especially under intensified cropping systems in greenhouse conditions. In the present research, the combined effects of biostimulant application and deficit irrigation on the biochemical characteristics and bioactive properties of tomato fruits were studied. Proximate composition was beneficially affected by full irrigation and biostimulant treatment (e.g., proteins and carbohydrates and energetic value were the highest for the XSW+ treatment), whereas fat and ash content were the highest for the CW+ and TWW- treatments. The irrigation regime and the biostimulant application also affected the free sugar composition, while organic acid and tocopherol content was beneficially affected by the TWW+ treatment. The content of the main fatty acids (palmitic, linoleic and oleic acids) varied depending on the applied treatment, while the highest values of β-carotene and lycopene were recorded for the CW- and NW+ treatments, respectively. Regarding the antioxidant activity, the TWW+ treatment showed high activity for both the tested assays (TBARS and OxHLIA), while most of the tested extracts showed lower antibacterial activity against the tested bacteria compared to the positive controls. On the other hand, the extracts obtained from the CW+, XSW+ and XSW- treatments showed higher antifungal activity (MIC values) than the positive controls. In conclusion, despite the positive effects recorded for specific biostimulant and irrigation combinations, more research is needed to better identify the mechanisms of action and the physiological processes, before the tested biostimulants can be used to standardize the application of such products in tomato cultivation. Moreover, the agronomic practice of deficit irrigation showed promising results in the sustainable management of natural resources in tomato crop, while its combined application with tailor-made biostimulants could be considered a viable water-saving technique without compromising the quality of tomato fruit.

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